

adhesion culture in each medium of Examples 5 and 6, and the 201B7 line was subjected to adhesion culture in each medium of Examples 4 to 6.

[0157] A 12-well plate coated with a basement membrane matrix (iMatrix-511 (Nippi, Inc.; 892012)) was prepared. Each medium (1 mL) of Examples 4 to 6 was added per well, 10,000 cells were seeded in the state of single cell, and cultured under the conditions of 37° C., carbon dioxide concentration=5% by volume, oxygen concentration=20% by volume for 7 days. To each medium was added Rho-associated kinase inhibitor (Y-27632) (Fuji Film Wako Pure Chemical Corporation; 034-24024) at a final concentration=10 μ M. The total amount of the medium was changed on days 1, 2, 5 and 6 of culture.

[0158] As a control, adhesion culture of iPS cell 1210B2 line and 201B7 line was performed in the same manner using StemFit (registered trade mark) AK03N medium (Ajinomoto Co., Inc.).

[0159] On day 7 of culture, the cell confluency was measured by IncuCyte (Essen BioScience K.K.). Adhesion culture and confluency measurement using each medium were performed 3 times each, and the measurement results are shown in FIGS. 2 and 3 in average values.

[0160] As shown in FIG. 2, in the adhesion culture of the 1210B2 line, improvement in cell proliferation ability was observed when each medium of Examples 5 and 6 of the present invention was used as compared with the control.

[0161] As shown in FIG. 3, in the adhesion culture of the 201B7 line, improvement in cell proliferation ability was observed when each medium of Examples 4 to 6 of the present invention was used.

[0162] From the above-mentioned results of Experimental Example 2, also in the adhesion culture of iPS cells, it was suggested that the cell proliferation ability is improved by the addition of five kinds of amino acids (L-tryptophan, L-serine, L-cysteine, L-methionine and L-arginine) or glucose, or the aforementioned five kinds of amino acids and glucose.

Examples 7 and 8

Suspension Culture of iPS Cells

[0163] Using 30 mL single-use bioreactor for iPS cells (ABLE Corporation: BWV-S03A), iPS cell 1210B2 line and 1231A3 line (iPS Academia Japan, Inc.) were seeded at a cell density of 6×10^5 cells/mL in StemFit (registered trade mark) AK03N medium (Ajinomoto Co., Inc.) supplemented with 10 μ M Rho-associated kinase inhibitor (Y-27632) (Fuji Film Wako Pure Chemical Corporation; 034-24024), and cultured by stirring in a CO₂ incubator (37° C., oxygen concentration=20% by volume, carbon dioxide concentration=5% by volume). On day 2 after seeding, 70% of the medium was exchanged with StemFit (registered trade mark) AK03N medium (Ajinomoto Co., Inc.). On day 3 after seeding, the cell suspension (10 mL) was resuspended in 10 mL of fresh StemFit (registered trade mark) AK03N medium (Ajinomoto Co., Inc.) (cell density= 0.806×10^6 cells/mL for 1210B2 line, cell density= 0.481×10^6 cells/mL for 1231A3 line), transferred into a micro bioreactor (ambr15 (Sartorius): 001-0881), and stirring culture was continued under the conditions of 37° C., pH=7.2, dissolved oxygen concentration=20% by volume, carbon dioxide concentration=5% by volume, stirring rate=300 rpm. 70% of the medium was exchanged once per day.

[0164] In this case, L-tryptophan (Ajinomoto Co., Inc.) (40 mg/L/day), L-serine (Ajinomoto Co., Inc.) (40 mg/L/day), L-cysteine hydrochloride (Nippon Protein Co., Ltd.) (40 mg/L/day), L-methionine (Ajinomoto Co., Inc.) (40 mg/L/day), L-arginine (Ajinomoto Co., Inc.) (160 mg/L/day), D-glucose (Nacalai Tesque Inc.; 16806-25) (4 g/L/day) were added to one group (Example 7), and the aforementioned five kinds of amino acids and glucose, as well as L-histidine (Ajinomoto Co., Inc.) (18.6 mg/L/day), L-isoleucine (Ajinomoto Co., Inc.) (43.7 mg/L/day), L-leucine (Ajinomoto Co., Inc.) (43.7 mg/L/day), L-lysine hydrochloride (Ajinomoto Co., Inc.) (73.1 mg/L/day), L-phenylalanine (Ajinomoto Co., Inc.) (28.4 mg/L/day) and L-valine (Ajinomoto Co., Inc.) (42.3 mg/L/day) were added to the other group and the cells were cultured (Example 8).

Experimental Example 3

Evaluation of Effect of the Culture Method of the Present Invention in Suspension Culture of iPS Cells

[0165] The number of viable cells was measured with a cell viability autoanalyzer (Vi-CELL (registered trade mark) XR (Beckman Coulter)) on day 8 of culture of 1210B2 line and on day 7 of culture of 1231A3 line.

[0166] The measurement results of viable cell number of 1210B2 line and 1231A3 line are shown in FIGS. 4 and 5, respectively.

[0167] As shown in FIGS. 4 and 5, the number of viable cells increased as compared with that when resuspended and the cell proliferation was promoted in both the 1210B2 line and 1231A3 line, both by culturing with addition of five kinds of amino acids (L-tryptophan, L-serine, L-cysteine hydrochloride, L-methionine and L-arginine) and glucose (Example 7), and by culturing with addition of 11 kinds of amino acids (L-tryptophan, L-serine, L-cysteine hydrochloride, L-methionine, L-arginine, L-histidine, L-isoleucine, L-leucine, L-lysine hydrochloride, L-phenylalanine and L-valine) and glucose (Example 8). A larger cell proliferation promoting effect was observed when 11 kinds of amino acids and glucose were added (Example 8).

INDUSTRIAL APPLICABILITY

[0168] As described in detail above, the present invention can provide an additive for culturing animal cells that can preferably improve the proliferation ability of animal cells, particularly stem cells, and can provide a medium for culturing animal cells and a method for culturing animal cells that can preferably improve the proliferation ability of animal cells.

[0169] Where a numerical limit or range is stated herein, the endpoints are included. Also, all values and subranges within a numerical limit or range are specifically included as if explicitly written out.

[0170] As used herein the words “a” and “an” and the like carry the meaning of “one or more.”

[0171] Obviously, numerous modifications and variations of the present invention are possible in light of the above teachings. It is therefore to be understood that, within the scope of the appended claims, the invention may be practiced otherwise than as specifically described herein.